AFFIDAVIT

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Successful production of influenza type A H5N1 vaccine

An avian influenza virus type A having an H5N1 was known previously to infect only birds. But H5N1 influenza A viruses are now considered to be of high pandemic potential as they can cross the avian-human species barrier and cause disease in humans. In August 1997 the WHO announced that H5N1 had been isolated in a 3 year-old boy who died 3 months earlier in Hong Kong. This was the first case of H5N1 infection in a human being. Direct avian-to-human influenza transmission was unknown before 1997. During the Hong Kong outbreak 18 persons were hospitalized with H5N1 influenza and six of them died.

The government of Vietnam started reporting hospitalized cases of severe respiratory illness in people from provinces surrounding Hanoi, which appeared to initiate in October 2003. On January 26, 2004 the Vietnamese government reported its first confirmed human cases in the south of the country, with two cases in Ho Chi Minh City. Beginning January 23, 2004, the government of Thailand began reporting avian influenza A infections as well. As of the writing date of this affidavit 42 deaths due to H5N1 have been reported in Thailand and Vietnam with one case from Cambodia. What is worrisome to all experts is that the mortality rate appears to be in the 70% range (42 fatalities out of 55 confirmed H5N1 infections). By comparison in 1997 Hong Kong outbreak the mortality rate was only 30%.

In addition to humans H5N1 virus also killed various other mammals, including leopards, tigers, mice, and domestic cats, suggesting that it can cross the barrier to infect mammalian species and may also spread between mammalian hosts.

The 1918 'Spanish flu' pandemic that took 40 million human lives is a vivid reminder of the possible death toll from new influenza strains. According to the WHO estimates even less virulent seasonal influenza epidemics results in three to five million cases of severe illness and between 250,000 to 500,000 deaths each year in the industrialized world alone.

An initial stock of H5N1 influenza virus was obtained from a Thai chicken farm and used to manufacture the first lot of H5N1 vaccine according to the method of the invention. As heterologous virus control H5N3 vaccine was also made. Viruses were grown in the allantoic cavities of 9-11-day old embryonated chicken eggs at 37 degrees for 24-48 hours. Allantoic fluid was harvested and used for vaccine preparation according to process described in specification of patent application.

The first lot of vaccine containing an equivalent of 15μg of HA per dose (established by the hemagglutination method) was tested for protection against H5N1 challenge in chickens. As a control we prepared a separate vaccine from the heterologous H5N3 virus. While this virus has shared a H5 determinant it has an unrelated neuraminidase subtype and should therefore provide a lesser protective effect.

Initial challenge experiments in a chicken model.

Chickens were administered orally one vaccine tablet (15 μg of HA per dose) once per day for seven days and challenged the eighth day with a lethal dose equivalent to $6.8x10^{6.5}$ TCID50 of H5N1 given intraorally in 0.1 ml solution. The infectious dose was established by titration in freshly isolated chicken lung fibroblasts. The results from the first test showed that all 3 unvaccinated chickens in the control group were dead within 30 hours. The group of chickens receiving the heterologous vaccine having H5N3 antigens had 40% fatalities by 30 hours. By comparison, the group of chickens receiving

vaccine to H5N1 influenza had 20% mortality at both 30 hours (Table 1) and at 42 hours (Not Shown). Based on these results it appeared that the protection conferred by H5N1 vaccine was 80%.

Table 1. Results from the first test at 30 hours post-infection

Intervention	Dead	Alive		Total	P
		Sick	Normal		
Control (■)	3	0	0	3	NA*
H5N3 (▼)	2	2	1	5	0.074
H5N1 (●)	1	2	2	5	0.028

*Not available since P value is assessed by χ^2 against control

Field experience

In October 2004 a local farmer who was the acquaintance of the inventors had obtained a bag of H5N1 pills. Having heard that it may prevent bird flu he decided to give pills to all his chickens. He gave two pills in one dose to ~50 adult chicken and one pill each to nine small chicks that just hatched. Inadvertently, about one week later there was as a flu outbreak at the surrounding farms that killed the entire chicken population in the neighborhood. As the chickens were bred in free-range style the disease spread to the farmer's birds. Within three days all adult chickens and 5 out of 9 chicks were dead. Although the results are preliminary this incident shows that a single oral dose of our vaccine is not effective in a field situation and that the efficacy is dose-related (Table 2).

Table 2. Survival of chicken fed with H5N1 pills prior to outbreak

Farm chicken	Dead	Alive	Total	P
Adults (2 pills) ■	50	0	50	NA*
Chicks (1 pill) o	5	4	9	<0.00001

Adult chickens that received 2 pills weighed about 2-3 kg each, but chicks weighed only 60-100g at the time when they were given a single pill. This suggests that the dose in the vaccine was sufficient to protect about half of the small-size chickens.

While protection observed in chicks is incomplete, i.e., 44%, this came as no surprise - so far the complete protection has been seldom observed with experimental avian flu vaccines reported by others. For example 50% protection was observed by Swayne et al. with their H5N1 vaccine. Also the post-vaccination timing for challenge appears to be critical. In a field study at two Hong Kong farms reported by Ellis et al., the infection was most likely to cause death in recently vaccinated chickens up to 18 days post-vaccination, but no deaths were observed after that period. Thus the level of protection conferred by our vaccine can likely be improved when the challenge timing and dosing factors are taken into consideration in proposed studies. Nevertheless this example shows that the influenza vaccine developed by the present inventors has shown efficacy in a real life situation and can indeed protect a portion of the chicken population from lethal challenge by this virulent influenza.

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5/6/05
Date